CLAIMS

1. A method for the preparation of cDNA tags for identifying expressed genes comprising:

providing complementary deoxyribonucleic acids (cDNAs);

cleaving the cDNAs with a type II restriction enzyme to prepare cDNA fragments;

ligating the cDNA fragments to linker Xes which have a recognition site of a first type IIS restriction enzyme and which form a recognition site of a second type IIS restriction enzyme at the site linking with the cleavage end-sites of the cDNA fragments formed by the type II restriction enzyme to prepare linker X-cDNA fragment complexs;

cleaving the linker X-cDNA fragment complexs with the second type II restriction enzyme to prepare linker X-cDNA tag complexs;

ligating linker Ys which have a recognition site of a third type IIS restriction enzyme to the cleavage end-sites of the linker X-cDNA tag complexs formed by the second type IIS restriction enzyme to prepare linker X-cDNA tag-linker Y complexs;

amplifying the linker X-cDNA fragment-linker Y complexs; and

cleaving the amplified products thus obtained with the first and third type IIS restriction enzymes simultaneously or in turn to prepare the cDNA tags for identifying expressed genes.

2. A method for the preparation of cDNA tags for identifying expressed genes comprising:

providing complementary deoxyribonucleic acids (cDNAs);

cleaving the cDNAs with a type II restriction enzyme to produce cDNA frangments;

ligating the cDNA fragments to linker Xes which have recognition sites of first and second type IIS restriction enzymes to prepare linker X-cDNA fragment complexs;

cleaving the linker X-cDNA fragment complexs with the second type IIS

restriction enzyme to prepare linker X-cDNA tag complexs;

ligating linker Ys which have a recognition site of the first type IIS restriction enzyme to the cleavage end-sites of the linker X-cDNA tag complexs formed by the second type IIS restriction enzyme to prepare linker X-cDNA tag-linker Y complexs;

amplifying the linker X-cDNA tag-linker Y complexs; and

cleaving the amplified products thus obtained with the first type IIS restriction enzyme to prepare the cDNA tags for identifying expressed genes.

3. A method for the preparation of cDNA tags for identifying expressed genes comprising:

providing complementary deoxyribonucleic acids (cDNAs);

cleaving the cDNAs with a type II restriction enzyme to produce cDNA frangments;

ligating the cDNA fragments to linker Xes which have recognition sites of first and second type IIS restriction enzymes to prepare linker X-cDNA fragment complexs;

cleaving the linker X-cDNA fragment complexs with the second type IIS restriction enzyme to prepare linker X-cDNA tag complexs;

ligating linker Ys which have a recognition site of a third type IIS restriction enzyme to the cleavage end-sites of the linker X-cDNA tag complexs formed by the second type IIS restriction enzyme to prepare linker X-cDNA tag-linker Y complexs;

amplifying the linker X-cDNA tag-linker Y complexs; and

cleaving the amplified products thus obtained with the first and third type IIS restriction enzymes simultaneously or in turn to prepare the cDNA tags for identifying expressed genes.

4. The method according to any one of claims 1, 2 or 3 further comprising the step of processing the cleavage end-sites of the cDNA fragments formed by the type II restriction enzyme to make the end-sites capable of binding to the linker Xes.

5. The method according to any one of claims 1, 2 or 3 further comprising the

step of refining the linker X-cDNA fragment complexs.

6. The method according to any one of claims 1, 2 or 3 further comprising the step of processing the end-sites of the cDNA fragments in the linker X-cDNA fragment complexs to make the end-sites capable of binding to the linker Ys.

7. The method according to claim 5 further comprising the step of processing the end-sites of the cDNA fragments in the linker X-cDNA fragment complexs to make the end-sites capable of binding to the linker Ys.

8. The method according to any one of claims 1, 2 or 3 further comprising the step of separating the obtained cDNA tags for identifying expressed genes.

9. The method according to any one of claims 1, 2 or 3 wherein the cDNAs are prepared from the mRNAs derived from cells to be examined.

10. The method according to any one of claims 1, 2 or 3 wherein the cDNAs are prepared from the mRNAs derived from cells to be examined using oligo-dT primers immobilized on a solid phase as an oligo-dT primer.

11. The method according to claim 10 wherein the oligo-dT primers comprise oligo-dT primers immobilized on latex beads or magnet beads.

12. The method according to any one of claims 1, 2 or 3 wherein the type II restriction enzyme has the recognition site of four base pairs.

13. The method according to any one of claims 1, 2 or 3 wherein the type II restriction enzyme is selected from the group consisting of AfaI, AluI, CfuI, CviRI, DpnI, EsaBC3I, HpyBI, HpyCH4V, HpyF44III, MltI, PlaAII, RsaI, BfaI, Csp6I, CviAII, CviQI, CviRII, FgoI, HpyCH4IV, MaeI, MaeII, MthZI, RmaI, PpaAII, Tsp32I, Tsp32II, TaqI, TthHB8I, XspI, BspKT6I, BstKTI, HpyCH4I, AspMDI, Bce243I, Bfi57I, BfuCI, Bme12I, BscFI, Bsp67I, Bsp105I, Bsp143I, Bsp2095I, BspAI, BspFI, BspJI,

Bst19II, BstENII, BtkII, CacI, CcyI, ChaI, CpfI, CviAI, DpnII, FatI, FnuCI, FnuEI, HacI, Kzo9I, LlaAI, MboI, MgoI, MkrAI, NdeII, NlaII, NmeCI, NphI, RalF40I, Sau3AI, SauMI, Sth368I, Hn1II, Hsp92II, NlaIII, TaiI, TscI and Tsp49I.

14. The method according to any one of claims 1, 2 or 3 wherein the first type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, Bsp423I, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII, BseRI, BspST5I, LweI, PhaI, SfaNI, AarI, Acc36I, BfuAI, BspMI, BveI, Sth132I, SspD5I, AsuHPI, HphI, MboII, NcuI, MnlI, BbsI, BbvII, BbsI, Bbv16II, BpiI, BpuAI, Bsc91I, BspBS31I, BspIS4I, BspTS514I, BstBS32I, BstTS5I, BstV2I, Bme585I, BscAI, Bst19I, BstFZ438I, FauI, SmuI, BciVI, BfuI and HpyAV.

15. The method according to any one of claims 1, 2 or 3 wherein the first type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, Bsp423I, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII and BseRI.

16. The method according to any one of claims 1, 2 or 3 wherein the first type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI and GsuI.

17. The method according to any one of claims 1, 2 or 3 wherein the second type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII, BseRI, BspST5I, LweI, PhaI, SfaNI, AarI, Acc36I, BfuAI, BspMI, BveI, Sth132I, SspD5I, AsuHPI, HphI, MboII, NcuI, MnlI, BbsI, BbvII, BbsI,

Bbv16II, BpiI, BpuAI, Bsc91I, BspBS31I, BspIS4I, BspTS514I, BstBS32I, BstTS5I, BstV2I, Bme585I, BscAI, Bst19I, BstFZ438I, FauI, SmuI, BciVI, BfuI and HpyAV.

18. The method according to any one of claims 1, 2 or 3 wherein the second type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII and BseRI.

19. The method according to any one of claims 1, 2 or 3 wherein the second type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI and GsuI.

20. The method according to any one of claims 1 or 3 wherein the third type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII, BseRI, BspST5I, LweI, PhaI, SfaNI, AarI, Acc36I, BfuAI, BspMI, BveI, Sth132I, SspD5I, AsuHPI, HphI, MboII, NcuI, MnlI, BbsI, BbvII, BbsI, Bbv16II, BpiI, BpuAI, Bsc91I, BspBS31I, BspIS4I, BspTS514I, BstBS32I, BstTS5I, BstV2I, Bme585I, BscAI, Bst19I, BstFZ438I, FauI, SmuI, BciVI, BfuI and HpyAV.

21. The method according to any one of claims 1 or 3 wherein the third type IIS restriction enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI, GsuI, BsmFI, BspLU11III, BstOZ616I, StsI, BceAI, BstPZ418I, FokI, BcefI, AlwXI, BbvI, BseKI, BseXI, Bsp423I, Bst12I, Bst71I, BstV1I, RleAI, AceIII, Bbr7I, EciI, TspDTI, TspGWI, Tth111II, HgaI, BseMII and BseRI.

22. The method according to claim 1 wherein the third type IIS restriction

enzyme is selected from the group consisting of MmeI, AcuI, Bce83I, BpmI, BpuEI, BsgI, BspKT5I, Eco57I, Eco57MI and GsuI.

23. The method according to any one of claims 1, 2 or 3 wherein the lengths of the cDNA tags for identifying expressed genes ranges from 6 base pairs (bp) to 25 bp.

24. The method according to any one of claims 1, 2 or 3 wherein the lengths of the cDNA tags for identifying expressed genes ranges from 10 bp to 25 bp.

25. The method according to any one of claims 1, 2 or 3 wherein the lengths of the cDNA tags for identifying expressed genes ranges from 10 bp to 16 bp.

26. Linker X comprising recognition sites of the first and second type IIS restriction enzymes.

27. Linker X according to claim 26 comprising base sequences of SEQs 7 and 8.

28. Linker X-cDNA fragment complex comprising cDNA fragment formed by cleaving with a type II restriction enzyme and linker X having recognition sites of the first and second type IIS restriction enzymes.

29. Linker X-cDNA tag-linker Y complex wherein linker Y having a recognition site of the third type IIS restriction enzyme is ligated at the cleavage end-site of linker X-cDNA fragment complex.

30. The linker X-cDNA tag-linker Y complex according to claim 29 comprising base sequence of SEQ 19 and its complementary sequence.

31. Library of cDNA tags for identifying expressed genes prepared by the method according to any one of claims 1, 2 or 3.

32. A method for the analysis of gene expression wherein the library of cDNA

tags according to claim 31 is contacted with a detector on which nucleic acids to be detected are immobilized.

33. The method for the analysis of gene expression according to claim 32 wherein the detector comprises DNA chip having spots on which nucleic acids to be detected are immobilized.

34. A method for the analysis of gene expression wherein a library of nucleic acids to be detected is connected with a detector on which cDNA tags prepared by the method according to any one of claims 1, 2 or 3 are immobilized.

35. The method according to claim 34 wherein the detector comprises a DNA chip having spots on which the cDNA tags prepared by the method according to any one of claims 1, 2 or 3 are immobilized.

36. A method for the analysis of gene expression comprising the steps of concatenating cDNA tags prepared by the method according to any one of claims 1, 2 or 3 each other to form concatemers and sequencing the concatemers.

37. The method according to claim 36 wherein the concatemer consists of 3 to 200 of the cDNA tags for identifying expressed genes.

38. The method according to claim 36 wherein the concatemer consists of 3 to 80 of the cDNA tags for identifying expressed genes.

39. The method according to claim 36 wherein the concatemer consists of 16 to 40 of the cDNA tags for identifying expressed genes.

40. The method for the qualitative analysis of gene expression according to claim 36 wherein the concatemers are sequenced and then each of the cDNA tags are sequenced on the basis of the sequences of the concatemers.

41. The method for the quantitative analysis of gene expression according to claim 36 wherein the concatemers are sequenced and then each of the cDNA tags are sequenced and measured in frequency of occurrences on the basis of the sequences of the concatemers.

42. A concatemer consisting of the cDNA tags prepared by the method according to any one of claims 1, 2 or 3 wherein no spacer sequence exists among the cDNA tags.

43. The concatemer according to claim 42, which consists of 3 to 200 of the cDNA tags.

44. The concatemer according to claim 42, which consists of 3 to 80 of the cDNA tags.

45. The concatemer according to claim 42, which consists of 16 to 40 of the cDNA tags.

46. A concatemer consisting of the cDNA tags prepared by the method according to any one of claims 1, 2 or 3 wherein spacer sequences exist among the cDNA tags.

47. The concatemer according to claim 46, which consists of 3 to 200 of the cDNA tags.

48. The concatemer according to claim 46, which consists of 3 to 80 of the cDNA tags.

49. The concatemer according to claim 46, which consists of 16 to 40 of the cDNA tags.

50. A kit for the preparation of cDNA tags for identifying expressed genes wherein the kit comprises a type II restriction enzyme, a first type IIS restriction

enzyme, a second type IIS restriction enzyme, a third type IIS restriction enzyme, linker Xes which have a recognition site of the first type IIS restriction enzyme and which form a recognition site of the second type IIS restriction enzyme at the site linking with the cleavage end-sites of the cDNA fragments formed by the type II restriction enzyme to prepare linker X-cDNA fragment complexs, and linker Ys which have a recognition site of a third type IIS restriction enzyme.

51. A kit for the preparation of cDNA tags for identifying expressed genes wherein the kit comprises a type II restriction enzyme, a first type IIS restriction enzyme, a second type IIS restriction enzyme, linker Xes which have recognition sites of the first and the second type IIS restriction enzymes, and linker Ys which have a recognition site of the first type IIS restriction enzyme.

52. A kit for the preparation of cDNA tags for identifying expressed genes wherein the kit comprises a type II restriction enzyme, a first type IIS restriction enzyme, a second type IIS restriction enzyme, a third type IIS restriction enzyme, linker Xes which have recognition sites of the first and second type IIS restriction enzymes, and linker Ys which have a recognition site of the third type IIS restriction enzyme.

53. The kit according to any one of claims 50, 51 or 52 wherein the kit comprises primer Xes which hybridize the linker Xes and primer Ys which hybridize linker Ys.